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Activity of eight antimicrobial agents *in vitro* against *N. gonorrhoeae*

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The occurrence of gonococcal strains which are relatively resistant to penicillin and other antibiotics has gradually come to be an important practical problem during the past 15 years. It is striking that the percentages of penicillin relatively resistant (PRR) strains found in Europe are always lower than those found in other parts of the world, being less than 40 per cent. in practically all investigations. In recent years, there have even been reports of a drop in the percentage of PRR strains in Europe. Alarmingly high percentages of PRR strains (>70 per cent.) have been found in Africa, the Far East, and the Pacific.

The percentages of PRR strains among the strains investigated in India, the USA, and Canada lie between those found in Europe and in Africa, the Far East, and the Pacific (Willcox, 1970, 1972; Wigfield, Selkon, and Rich, 1973). The favourable situation in Europe could be unfavourably influenced by the importing of PRR strains. Sailors form one group which could be responsible for importing them (Wols- van der Wielen, 1971).

Strong positive rank correlations have been found between the distributions of the sensitivities of gonococcal strains to antibiotics with such widely differing chemical structures and modes of operation as penicillin, tetracycline, erythromycin, chloramphenicol, streptomycin, fusidinic acid, and rifampicin (Reyn, 1961; Reyn and Bentzon, 1968, 1969; Phillips, Rimmer, Ridley, Lynn, and Warren, 1970; Maness and Sparling, 1973). Reyn and Bentzon (1969) stated that these correlations were strongest at the highest levels of (relative) resistance observed for each drug. Only partial or relative resistance has been found for all antibiotics with the exception of streptomycin and spectinomycin.

This study describes quantitative determinations of the sensitivities of four groups of gonococcal strains to ampicillin (Am), penicillin (P), tetracycline (T), rifampicin (Ri), spectinomycin (Sp), sulphamethoxazole (Su), trimethoprim (Tr), and a combination of Su and Tr in the ratio 5:1 (Su/Tr = 5:1). The various groups of strains were isolated in Rotterdam (R) in 1972 from male civilians (MC), female civilians (FC), and sailors (S) by the authors, and in Amsterdam (A) by Heimans (1967) from FC. This made it possible to compare the S-R strains (isolated from sailors in Rotterdam), which may be regarded as largely imported, with the MC-R and FC-R strains which may be regarded as mainly of Dutch origin. Furthermore, a comparison of the FC-A and FC-R strains will show up any changes in the frequency of (relative) resistance to the antibiotics investigated in the Netherlands from 1967 to 1972. Finally, rank correlations between the sensitivity distributions for all pairs of antibiotics are calculated for all groups of strains and discussed.

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Material and Methods

Quantitative sensitivity determinations for Am, P, T, Ri, Tr, and Su/Tr 5:1 were carried out from May 15 to July 28, 1972, on 248 strains of *N. gonorrhoeae* isolated in the outpatient department for Dermatology and Venereal Diseases of the University Hospital, Rotterdam. Of the 248 strains, isolated from 248 patients, 114 were from MC, 90 from FC, and 44 from S. The majority of these strains, *viz.* those isolated from May 15 to June 30, were also included in an earlier study on sensitivity testing for Am, P, and T (Stolz, Zwart, and Michel, 1974).

In addition quantitative sensitivity determinations for all the above mentioned antibiotics were performed on 94 freeze-dried FC-A strains of *N. gonorrhoeae* isolated by Heimans (1967).

Sensitivity was expressed as the minimum inhibitory concentration (MIC) in μg./ml. Sensitivity testing was performed by the agar dilution method (Ericsson and Sherris, 1971). The medium used for sensitivity testing for Am, P, T, Ri, and Sp was Bacto GC Medium Base (Difco 0289) + Bacto haemoglobin (Difco 0136) + Isovitalex TM Enrichment (BBL 11878). The medium used for sensitivity testing for Su, Tr, and Su/Tr 5:1 was DST agar (Oxford CM 261) + 7·5 per cent. haemolysed horse blood.

A series of plates with increasing concentrations of the antibiotic and one control plate without antibiotic were prepared. The concentrations of Am were in the range 0·005–1·28 μg./ml., those of P 0·0025–1·28 μg./ml., those of T 0·01–5·12 μg./ml., those of Ri 0·0025–0·64 μg./ml. those of Sp 7·5–25·0 μg./ml., those of Su 0·16–20·48 μg.

ml., those of Tr 2·56–40·96 μg./ml., and those of Su/Tr 5:1 0·10–12·29 μg./ml.

The organisms to be tested were freshly suspended in trypticase soy broth (TSB) (BBL 11768) to a density of 10^6–10^7 v.u./ml. These suspensions were inoculated on the plate series with a multipoint replicator, resulting in spot inocula covering a circle of 4–6 mm. diameter and containing 10^3–10^4 v.u.

In each run, three gonococcal strains and one *Staphylococcus aureus* (Oxford strain) with known sensitivities for Am, P, and T were tested simultaneously. After incubation (18–20 hrs) the MIC was determined by reading off the lowest concentration of antibiotic at which bacterial growth was completely or almost completely inhibited, as judged by the naked eye. A haze of growth or a single colony was disregarded.

The results were recorded on optically readable forms. With the aid of an IBM 1232 mark page-reader, the forms were converted into punched cards by a coupled IBM 534 card punch. With the assistance of the System Development Department of the Medical Faculty, use was made of an IBM 2780 terminal connected *via* a permanent telephone line to an IBM 360 (model 65) computer system.

Results

The distribution of the MICs for the eight antibiotics investigated are given for the MC-R, FC-R, S-R, and FC-A strains as absolute values in Tables I to IV and as percentages of the total number of strains investigated in Tables I to IV and Figs 1 to 4. The figures are discussed here in semi-qualitative terms; for more exact information, the reader is referred to the Tables. Fig. 1 gives the MIC distributions for Am, P, T, and Ri; Fig. 2 for Sp, Fig. 3 for Su and Tr, and Fig. 4 for Su/Tr 5:1.

The MIC distributions for Am and P (Fig. 1) are bimodal, the left peak being more pronounced than the right peak for the MC-R, FC-R, and FC-A strains. For the S-R strains, the dip between the peaks lies one concentration step further to the right than for the other strains, and the right peak is more pronounced than the left.

The MIC distributions for T (Fig. 1) for the MC-R, FC-R, and FC-A strains are characterized by a peak at the left-hand end of the histogram, while the S-R strains give a bimodal distribution with the left peak practically as high as the right one.

The MIC distributions for Ri (Fig. 1), for the MC-R and FC-R strains show a peak sloping off stepwise to the right, with a maximum at 0·08 μg./ml.; that for the FC-A strains has a peak sloping off stepwise to both left and right, with a maximum at 0·08 and 0·16 μg./ml.; the peak for the S-R strains is block-shaped and covers the MICs 0·08, 0·16, and 0·32 μg./ml.

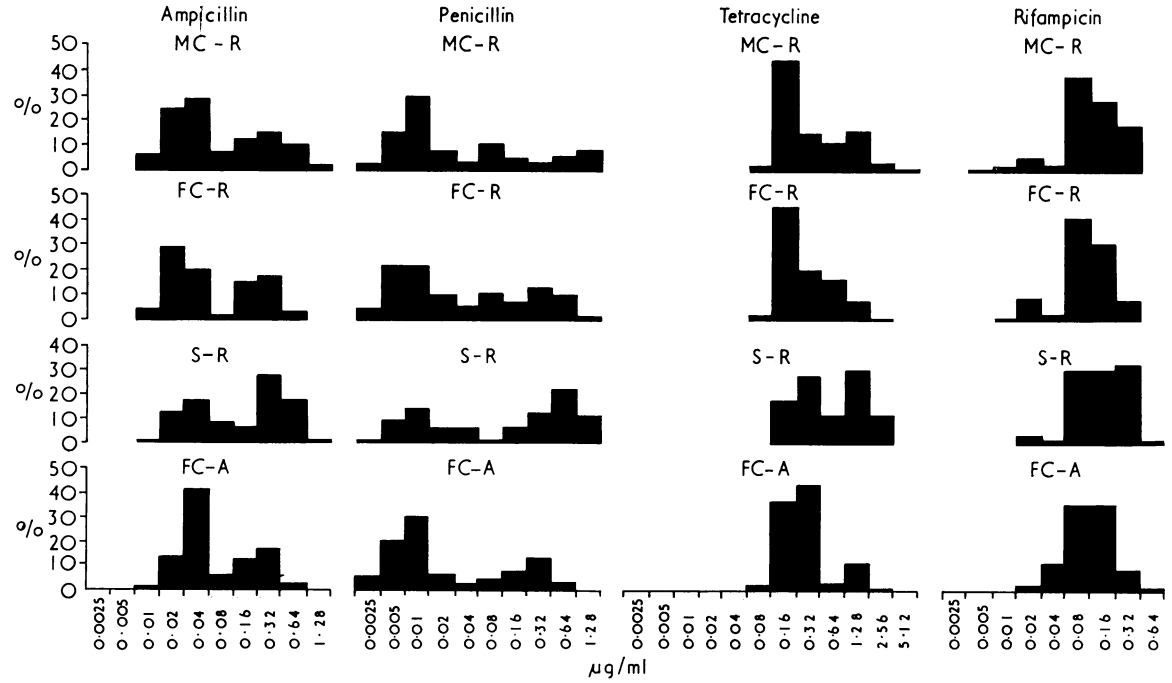

FIG. 1 *Distribution of MICs, presented as percentages of total number of strains investigated, for ampicillin, penicillin, tetracycline, and rifampicin of strains of gonococci isolated in* 1972 *from male civilians (MC), female civilians (FC), and sailors (S) in Rotterdam (R), and in* 1967 *from female civilians (FC) in Amsterdam (A)*

TABLE I *Distribution of MIC's for ampicillin (Am), penicillin (P), tetracycline (T), and rifampicin (Ri) of strains of gonococci isolated in* 1972 *from male civilians (MC), female civilians (FC), and sailors (S) in Rotterdam (R), and in* 1967 *from female civilians (FC) in Amsterdam (A)*

					MIC (μg./ml.)											
Antibiotic	*Population*	*No. of strains*		*Not performed*	0·0025	0·005	0·01	0·02	0·04	0·08	0·16	0·32	0·64	1·28	2·56	5·12
Am	MC-R	114	No.	2	—	—	7	27	31	7	12	16	11	1	—	—
			%	—	—	—	6·3	24·1	27·7	6·3	10·7	14·3	9·8	0·9	—	—
	FC-R	80	No.	1	—		4	23	17	2	14	15	4	—	—	—
			%	—	—		5·1	29·1	21·5	2·5	17·7	19·0	5·1	—	—	—
	S-R	44	No.	1	—	—	1	6	8	4	3	12	8	1	—	—
			%	—	—	—	2·3	14·0	18·6	9·3	7·0	27·9	18·6	2·3	—	—
	FC-A	94	No.	—	—		2	14	39	7	12	17	3	—	—	—
			%	—	—		2·1	14·9	41·5	7·4	12·8	18·1	3·2	—	—	—
P	MC-R	114	No.	2	4	17	35	9	5	13	6	5	8	10	—	—
			%	—	3·6	15·2	31·3	8·0	4·5	11·6	5·4	4·5	7·1	8·9	—	—
	FC-R	80	No.	1	3	17	17	8	3	8	5	11	6	1	—	—
			%	—	3·8	21·5	21·5	10·1	3·8	10·1	6·3	13·9	7·6	1·3	—	—
	S-R	44	No.	1	1	4	7	3	3	1	3	6	10	5	—	—
			%	—	2·3	9·3	16·3	7·0	7·0	2·3	7·0	14·0	23·3	11·6	—	—
	FC-A	94	No.	—	6	20	28	7	3	5	8	13	4	—	—	—
			%	—	6·4	21·3	29·8	7·4	3·2	5·3	8·5	13·8	4·3	—	—	—
T	MC-R	114	No.	2	—	—	—	—	—	3	52	19	14	19	4	1
			%	—	—	—	—	—	—	2·7	46·4	17·0	12·5	17·0	3·6	0·9
	FC-R	80	No.	1	—	—	—	—	—	2	37	17	14	8	1	—
			%	—	—	—	—	—	—	2·5	46·8	21·5	17·7	10·1	1·3	—
	S-R	44	No.	1	—	—	—	—	—	—	8	12	5	13	5	—
			%	—	—	—	—	—	—	—	18·6	27·9	11·6	30·2	11·6	—
	FC-A	94	No.	—	—	—	—	—		2	35	41	4	11	1	—
			%	—	—	—	—	—		2·1	37·2	43·6	4·3	11·7	1·1	—
Ri	MC-R	114	No.	—	—	1	3	7	5	43	33	22	—	—	—	—
			%	—	—	0·9	2·6	6·1	4·4	37·7	28·9	19·3	—	—	—	—
	FC-R	80	No.	3	—	—	1	8	3	33	24	8	—	—	—	—
			%	0	—	—	1·3	10·4	3·9	42·9	31·2	10·4	—	—	—	—
	S-R	44	No.	—	—	—	—	2	1	13	13	14	1	—	—	—
			%	—	—	—	—	4·5	2·3	29·5	29·5	31·8	2·3	—	—	—
	FC-A	94	No.	5	—	—	—	2	12	33	33	8	1	—	—	—
			%	—	—	—	—	2·2	13·5	37·1	37·1	9·0	1·1	—	—	—

The MIC distributions for Sp (Fig. 2), are U-shaped for the MC-R and FC-R strains, covering MICs from 15 to 20 μg./ml; that for the S-R strains shows a peak which slopes off stepwise to left and right, with a maximum at 17·5 μg./ml.; and that for the FC-A strains has a peak sloping off stepwise to the left with a maximum at 20 μg./ml. However, in measuring the MICs for Sp we did not use concentrations in a logarithmic scale. Only one (FC-A) strain had an MIC greater than 25 μg./ml. (Table II).

The MIC distributions for Su (Fig. 3) for the MC-R, FC-R, and S-R strains are characterized by peaks of various forms, covering values from 1·28 to 10·24 μg./ml. The peak for the FC-A strains extends further left in the diagram.

The MIC distribution for Tr (Fig. 3) for the MC-R strains is characterized by a peak sloping off stepwise to the right, with a maximum at 7·68 and 10·24 μg./ml.

The MIC distributions for Tr (Fig. 3) for the FC-R, S-R, and FC-A strains are characterized by peaks with a maximum at 15·36 and 20·48 μg./ml. For the FC-R and FC-A strains there are more to the left than to the right of the peak, while for the S-R strains there are more to the right than to the left.

The MIC distribution for Su/Tr (Fig. 4) is characterized by peaks at 1·54 and 3·07 μg./ml. for all strains. For the MC-R, FC-R, and S-R strains, there are roughly the same number to the left and right of the peak, and with the FC-A strains there appear to be more on the left.

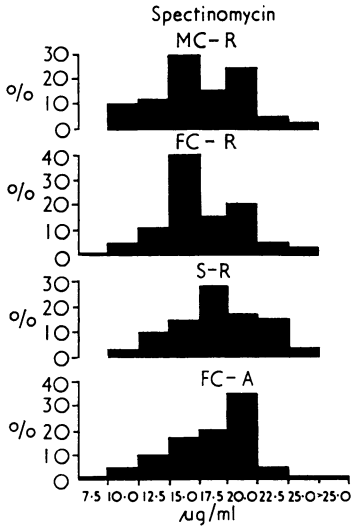

FIG. 2 *Distribution of MICs for spectinomycin of strains of gonococci isolated in* 1972 *from male civilians (MC), female civilians (FC), and sailors (S) in Rotterdam (R), and in* 1967 *from female civilians (FC) in Amsterdam (A)*

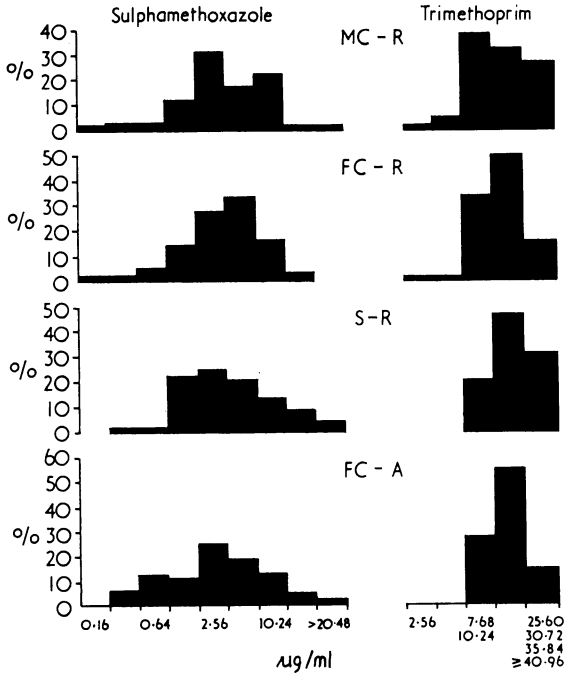

FIG. 3 *Distribution of MICs for suphamethoxazole and trimethoprim of strains of gonococci isolated in* 1972 *from male civilians (MC), female civilians (FC), and sailors (S), in Rotterdam (R), and in* 1967 *from female civilians (FC) in Amsterdam (A)*

The percentages of the MC-R, FC-R, S-R, and FC-A strains which are relatively resistant to Am, P, T, and Ri are presented in Table V.

The S-R group gave the highest percentages of strains relatively resistant to Am, P, T, and Ri. The percentages relatively resistant to Am, P, T, and Ri were compared with the aid of the χ^2 (1 d.f.; $P=0{\cdot}05$) on the observed frequencies for the strains MC-R and FC-R, MC-R and S-R, FC-R and S-R, and FC-R and FC-A. S-R and FC-A strains were not compared. Significant differences were found only for Am, P, and T between the MC-R and S-R strains, and for T and Ri between the FC-R and S-R strains.

For Sp, Su, Tr, and Su/Tr 5:1, the position of the boundary between sensitive and (relatively) resistant strains is not known. Moreover, the χ^2 test represents a very rough approximation when applied to the distribution of sensitivity and relative resistance to Am, P, T, and Ri in the various strains.

For this reason, we used the Yates–Cochran test with continuity correction to test for significant

TABLE II *Distribution of the MICs for spectinomycin of strains of gonococci isolated in* 1972 *from male civilians (MC), female civilians (FC), and sailors (S) in Rotterdam (R), and in* 1967 *from female civilians (FC) in Amsterdam (A)*

Population	*No. of strains*		*MIC (µg./ml.)*									
			Not performed	7·5	10·0	12·5	15·0	17·5	20·0	22·5	25·0	>25·0
MC-R	114	No.	1	—	11	13	33	17	28	6	5	—
		%	—	—	9·7	11·5	29·2	15·0	24·8	5·3	4·4	—
FC-R	80	No.	4	1	4	8	30	11	15	4	3	—
		%	—	1·3	5·3	10·5	39·5	14·5	19·7	5·3	3·9	—
S-R	44	No.	—	—	2	5	7	13	8	7	2	—
		%	—	—	4·5	11·4	15·9	29·5	18·2	15·9	4·5	—
FC-A	94	No.	6	1	5	10	16	18	31	5	1	1[a]
		%	—	1·1	5·7	11·4	18·2	20·5	35·2	5·7	1·1	1·1

[a] = MIC > 168µg./ml.

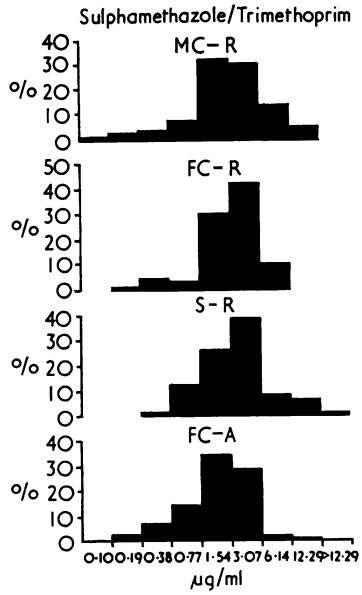

FIG. 4 *Distribution of MICs for combination of suphamethoxazole and trimethoprim in a proportion of* 5:1 *of strains of gonococci isolated in* 1972 *from male civilians (MC), female civilians (FC), and sailors (S), in Rotterdam (R), and in* 1967 *from female civilians (FC) in Amsterdam (A)*

differences in the MIC distributions for the various antibiotics between the strains MC-R and FC-R, MC-R and S-R, FC-R and S-R, and FC-R and FC-A. Significant differences were found between MC-R and S-R and between FC-R and S-R for Am, P, T, Ri, and Tr. In all these cases, the S-R strains were the least sensitive (see Table VI).

Furthermore, the FC-A strains were significantly more sensitive to Su/Tr 5:1 than the FC-R strains (means $X_1 = 4{\cdot}5$; $X_2 = 4{\cdot}0$; standard value $T = 2{\cdot}6$; two-sided significance $P = 0{\cdot}009$).

Table VII gives Spearman's rank correlation coefficient r for the MIC distributions of the various pairs of antibiotics investigated, for the strains MC-R FC-R, S-R, and FC-A. For the sake of simplicity, we shall refer briefly henceforward to the r's between two antibiotics instead of to the r's of the MIC distributions for the pair of antibiotics in question.

The value of r between Am and P, Am and T, and P and T was found to be $\geqslant 0{\cdot}50$ for all groups of strains. Between Am and Ri, P and Ri, and Am and Tr, $r \geqslant 0{\cdot}50$ was found only for S-R. In addition, the value of r between Ri and T, Tr and P, Tr and T, and Tr and Ri was higher for the S-R strains than for the MC-R and FC-R strains. Appreciably higher values of r (though not $\geqslant 0{\cdot}50$) were found between Am and Sp, P and Sp, T and Sp, and Ri and Sp for the FC-A strains than for the other groups of strains. For the MC-R and S-R strains, we found $r \geqslant 0{\cdot}50$ between Su and Tr, Su and Su/Tr 5:1, and Tr and Su/Tr 5:1. For the FC-R strains, r between Su and Tr was 0·35, and that between Su and Su/Tr 5:1 and Tr and Su/Tr 5:1 was $\geqslant 0{\cdot}50$, while for the FC-A strains we found $r \geqslant 0{\cdot}50$ between

TABLE III *Distribution of MICs for sulphamethoxazole (Su) and trimethoprim (Tr) of strains of gonococci isolated in* 1972 *from male civilians (MC), female civilians (FC), and sailors (S), in Rotterdam (R), and in* 1967 *from female civilians (FC) in Amsterdam (A)*

Drug	*Population*	*No. of strains*		*MIC (μg./ml.)*														
				Not performed	0·16	0·32	0·64	1·28	2·56	5·12	7·68	10·24	15·36	20·48	25·60	30·72	35·84	⩾40·96 >20·48 *SuMIC*
Su	MC-R	114	No.	4	3	4	4	13	35	19	—	26	—	3	—	—	—	3
			%	—	2·7	3·6	3·6	11·8	31·8	17·3	—	23·6	—	2·7	—	—	—	2·7
	FC-R	80	No.	2	1	1	4	11	21	26	—	12		2	—	—	—	—
			%	—	1·3	1·3	5·1	14·1	26·9	33·3	—	15·4		2·6	—	—	—	—
	S-R	44	No.	—	—	1	1	10	11	9	—	6	—	4	—	—	—	2
			%	—	—	2·3	2·3	22·7	25·0	20·5	—	13·6	—	9·1	—	—	—	4·5
	FC-A	94	No.	4	—	6	12	11	23	17	—	13	—	5	—	—	—	3
			%	—	—	6·7	13·3	12·2	25·6	18·9	—	14·4	—	5·6	—	—	—	3·3
Tr	MC-R	114	No.	4	—	—	—	—	2	5	18	24	19	16	9	6	10	1
		—	—	% —	—	—	—		1·8	4·5	16·4	21·8	17·3	14·5	8·2	5·5	9·1	0·9
	FC-R	80	No.	2	—	—	—	—	1	1	8	19	18	20	3	6	1	1
			%	—	—	—	—	—	1·3	1·3	10·3	24·4	23·1	25·6	3·8	7·7	1·3	1·3
	S-R	44	No.	—	—	—	—	—	—	—	4	5	6	15	5	2	1	6
			%	—	—	—	—	—	—	—	9·1	11·4	13·6	34·1	11·4	4·5	2·3	13·6
	FC-A	94	No.	4	—	—	—	—	—	—	11	16	32	17	5	4	5	—
			%	—	—	—	—	—	—	—	12·2	17·8	35·6	18·9	5·6	4·4	5·6	—

TABLE IV *Distribution of MICs for combination of sulphamethoxazole (Su) and trimethoprim (Tr) in a proportion of* 5:1 *(Su/Tr* 5:1*) of strains of gonococci isolated in* 1972 *from male civilians (MC), female civilians (FC). and sailors (S) in Rotterdam (R), and in* 1967 *from female civilians (FC) in Amsterdam (A)*

Population	*No. of strains*		*MIC (μg./ml.)*									
			Not performed	0·10	0·19	0·38	0·77	1·54	3·07	6·14	12·29	>12·29
MC-R	114	No.	4	1	3	5	10	36	33	16	6	—
		%	—	0·9	2·7	4·5	9·1	32·7	30·0	14·5	5·5	—
FC-R	80	No.	2	—	1	5	4	24	35	9	—	—
		%	—	—	1·3	6·4	5·1	30·8	44·9	11·5	—	—
S-R	44	No.	—	—	—	1	6	12	17	4	3	1
		%	—	—	—	2·3	13·6	27·3	38·6	9·1	6·8	2·3
FC-A	94	No.	4	—	4	8	14	32	27	3	2	—
		%	—	—	4·4	8·9	15·6	35·6	30·0	3·3	2·2	—

TABLE V *Percentages of strains relatively resistant (RR) to ampicillin (Am), penicillin (P), tetracycline (T), and rifampicin (Ri) among strains of gonococci isolated in* 1972 *from male civilians (MC), sailors (S), and female civilians (FC) in Rotterdam (R), and in* 1967 *from FC in Amsterdam (A)*

Population	*MC-R*	*S-R*	*FC-R*	*FC-A*
RR to Am (MIC ⩾ 0·16 μg./ml.)	35·7——55·8		41·8	34·1
RR to P (MIC ⩾ 0·08 μg./ml.)	37·5——58·2		39·2	31·9
RR to T (MIC ⩾ 1·28 μg./ml.)	21·5——41·8——		11·4	12·8
RR to Ri (MIC ⩾ 0·32 μg./ml.)	19·3	34·1——	10·4	10·1

—— = significant difference (χ^2 test; 1 d.f.; P = 0·05).

Su and Su/Tr 5:1 only; the *r*s between Su and Tr and between Tr and Su/Tr 5:1 were low (0·11 and 0·14 respectively).

Discussion

Reyn, Korner, and Bentzon (1958) compared the distributions of the sensitivities to P of gonococcal strains isolated in 1944 and 1957. The strains isolated in 1944 showed a unimodel distribution of sensitive strains, and those isolated in 1957 showed a bimodal distribution, with one peak for sensitive strains and one for relatively resistant strains.

We also found bimodal distributions for the MICs for Am and P (Fig. 1). Comparison of the S-R strains with the MC-R and FC-R reveal the more pronounced right-hand peak in the case of S-R. The (relative) resistance to T and Tr is not as marked in the MC-R and FC-R strains as that to Am and P; however, the S-R strains show a clear bimodal distribution of T-sensitive and R-relatively resistant strains. The higher percentage of strains RR to Ri and Tr in the histogram for the S-R strains is reflected not in a bimodal distribution but in an overall shift of the whole curve to the right (Figs 1 and 3). The MIC distributions for Am, P, T, Ri, and Tr

TABLE VI *Significant differences in distribution of MICs for Am, P, T, Ri, and Tr between strains of gonococci isolated in* 1972 *in Rotterdam (R), from male civilians (MC), and sailors (S), and between gonococcal strains isolated in* 1972 *in Rotterdam from female civilians (FC), and sailors (S) (Yates–Cochran test with continuity correction)*

Antibiotic	*Comparison between strains isolated from*	*X1*[a]	*X2*[a]	*T*[a]	*Two-sided significance*
Ampicillin	MC-R and S-R	2·8	3·7	2·8	0·006
	FC-R and S-R	2·8	3·7	2·7	0·007
Penicillin	MC-R and S-R	3·8	5·3	3·0	0·003
	FC-R and S-R	3·7	5·3	3·1	0·002
Tetracycline	MC-R and S-R	3·2	3·9	2·9	0·004
	FC-R and S-R	2·9	3·9	3·9	0·001
Rifampicin	MC-R and S-R	5·4	5·9	2·2	0·03
	FC-R and S-R	5·2	5·9	2·9	0·004
Trimethoprim	MC-R and S-R	4·1	5·2	2·8	0·006
	FC-R and S-R	4·1	5·2	2·9	0·003

X1 = mean of observations for first group of strains in col. 2.
X2 = mean of observations for second group of strains in col. 2.
T = standard value.
Difference between X1 and X2 considered to be significant when the two-sided significance (last column) is <0·05.
[a] = figures rounded off.

TABLE VII *Rank correlation coefficients between MIC distributions for various pairs of antibiotics investigated for strains isolated from MC-R, FC-R, S-R, and FC-A*

		Ampicillin						
Penicillin	MC-R	0·91						
	FC-R	0·94						
	S-R	0·91						
	FC-A	0·87	*Penicillin*					
Tetracycline	MC-R	0·63	0·70					
	FC-R	0·58	0·62					
	S-R	0·72	0·81					
	FC-A	0·55	0·61	*Tetracycline*				
Rifampicin	MC-R	0·27	0·27	0·23				
	FC-R	0·13	0·15	0·13				
	S-R	0·62	0·59	0·49				
	FC-A	0·38	0·43	0·38	*Rifampicin*			
Spectinomycin	MC-R	0·25	0·19	0·26	0·24			
	FC-R	0·13	0·08	0·06	0·10			
	S-R	0·07	0·05	0·04	0·14			
	FC-A	0·43	0·43	0·49	0·43	*Spectinomycin*		
Sulphamethoxazole	MC-R	0·08	0·13	0·15	0·05	0·09		
	FC-R	0·09	0·10	0·002	0·12	0·04		
	S-R	0·25	0·23	0·10	0·09	0·06		
	FC-A	0·04	0·02	0·02	0·12	0·27	*Sulphamethoxazole*	
Trimethoprim	MC-R	0·21	0·17	0·12	0·36	0·12	0·56	
	FC-R	0·26	0·30	0·26	0·23	0·03	0·35	
	S-R	0·50	0·44	0·37	0·49	0·09	0·52	
	FC-A	0·26	0·24	0·01	0·14	0·16	0·11	*Trimethoprim*
Sulphamethoxazole/Trimethoprim	MC-R	0·03	0·03	0·09	0·20	0·05	0·83	0·63
	FC-R	0·17	0·19	0·05	0·20	0·07	0·82	0·55
	S-R	0·15	0·15	0·10	0·06	0·15	0·78	0·53
	FC-A	0·07	0·17	0·10	0·05	0·24	0·84	0·14

in the S-R strains indicate the direction in which the (relative) resistance could develop in the MC-R and FC-R strains (Figs. 1 and 3). It was shown with the aid of the Yates-Cochran test with continuity correction that the S-R strains are significantly less sensitive to Am, P, T, Ri, and Tr than the MC-R and FC-R strains. This finding (insofar as Am, P, and T are concerned) is in agreement with previous observations (Stolz and others, 1974).

Comparison of the MIC distributions for Am, P, T, Ri, Sp, Su, and Tr in the FC-A strains (isolated in 1967) and the FC-R strains (isolated in 1972) revealed no marked changes (see Figs 1 to 3). However, one Sp-resistant strain was found in the FC-A group, with an MIC > 168 μg./ml. (Table II). Reyn, Schmidt, Trier, and Bentzon (1973) have described three Sp-resistant strains, with an $IC_{50} > 480$ μg./ml.

The only marked difference between the FC-A and FC-R strains was found in the MIC distributions for Su/Tr 5:1 (Fig. 4). It was shown with the aid of the Yates–Cochran test with continuity correction that the FC-A strains were significantly more sensitive to Su/Tr 5:1 than the FC-R strains. This is surprising, in view of the fact that no increase in the (relative) resistance to the other antibiotics investigated (in particular to Su and Tr) was found among Dutch FC-strains in the period from 1967 to 1972.

The potentiation of Su by Tr and hence the MIC for Su/Tr 5:1 is mainly determined by the ratio of the MIC for Su to that for Tr. This potentiation is absent or slight for strains in which this ratio is less than 1, is greater for strains in which the ratio is unity, and is greatest for the strains with a ratio > 1 (Stolz, 1974).

The difference between the FC-A and FC-R strains is due to the difference between the combinations of the MIC for Su and the MIC for Tr for the various gonococcal strains, and hence to the difference in the distributions of the ratio of the MIC for Su to the MIC for Tr. The fact that the value of r between Su and Tr is higher for the FC-R strains (0·35) than for the FC-A strains (0·11) also points in this direction. Table VII shows that there is a strong coupling between sensitivity or reduced sensitivity to Su, Tr, and Su/Tr 5:1 among the MC-R, FC-R, and S-R strains. In the FC-A strains, on the other hand, a high r is only found between Su and Su/Tr 5:1 and the r's between Su and Tr and between Tr and Su/Tr 5:1 are low. The higher degree of correlation among the Rotterdam strains could be due to the use of drugs containing Su and Tr (Bactrimel from Hoffmann-La Roche, and Eusaprim from Wellcome) in the years between 1967 and 1972.

In all groups of strains, r's $\geqslant 0{\cdot}50$ were found between Am and P, Am and T, and P and T. The

correlations found are in agreement with the results of an earlier study (Stolz and others, 1974). The values of *r* between Am and Ri, Am and Tr, P and Ri, P and Tr, T and Ri, T and Tr, and Ri and Tr for the S-R strains were higher than those for the MC-R and FC-R strains. This is in agreement with the observation reported by Reyn and Bentzon (1969) that the *r's* are highest at the highest levels of observed sensitivity for each drug.

Although the *r's* between Sp and the other antibiotics were highest in the FC-A strains and much lower in the R strains (in particular the S-R strains), little importance can be attached to this fact, since with one exception all strains were sensitive to Sp, with MICs between 7·5 and 25 μg./ml. The fact that none of the strains isolated in Rotterdam in 1972 was resistant to Sp indicates that Sp can be regarded as a reliable substitute for the drugs generally used at present for gonorrhoea therapy.

It follows from this study that, with the exception of the increased resistance to Su/Tr 5:1, there has been no significant change in the incidence of (relative) resistance to the antibiotics investigated among the Dutch civilian population in the period from 1967 to 1972. If the Rotterdam sailor strains (which are less sensitive to Am, P, T, Ri, and Tr than the male and female Rotterdam civilian strains) had been able to spread freely among the civilian population, there would have been an increase in the number of strains resistant to Am, P, T, Ri, and Tr among the civilian strains. It seems likely, however, that the S-R strains are mainly spread throughout the world *via* sailors and sailors' prostitutes. Spread of the sailor strains to the civilian population occurs only incidentally. Apart from this factor, efficient administration and the use of the right dosage in the therapy of gonorrhoea most probably plays an important role in keeping the percentage of strains (relatively) resistant to penicillin and other antibiotics among the civilian strains low in the Netherlands and other European countries.

Summary

The sensitivity of four groups of gonococcal strains to ampicillin (Am), penicillin (P), tetracycline (T), rifampicin (Ri), spectinomycin (Sp), sulphamethoxazole (Su), trimethoprim (Tr), and a combination of Su and Tr in the ratio 5:1 (Su/Tr 5:1) has been determined. The various groups of strains were isolated from male civilians (MC), female civilians (FC), and sailors (S) in Rotterdam (R) in 1972, and from FC in Amsterdam (A) in 1967. The S-R strains, which may be regarded as mainly imported, were compared with the MC-R and FC-R (which may be considered as mainly of Dutch origin). Also investigated was whether the incidence of relative resistance to the antibiotics investigated among Dutch strains has changed in the period from 1967 to 1972. For this purpose, the FC-A and FC-R strains were compared. The MC-R and FC-R strains were also compared with one another. Finally, Spearman's rank correlation coefficients *r* were calculated between the sensitivity distributions for each pair of antibiotics investigated, for all strains.

The S-R strains were significantly less sensitive to Am, P, T, Ri, and Tr than the MC-R and FC-R strains. Comparison of the FC-A and FC-R strains revealed that the FC-R strains were significantly less sensitive only to Su/Tr 5:1. A possible explanation for this finding is given.

With the exception of one FC-A strain, all gonococcal strains were sensitive to Sp.

High values of *r* ($\geqslant 0{\cdot}50$) were found between Am and P, Am and T, and P and T for all groups of strains. The values of *r* between any pair of the antibiotics Am, P, T, Ri, and Tr (with the exception of the pair Am-P) were always highest for the S-R strains.

High values of *r* ($\geqslant 0{\cdot}50$) were found between Su and Su/Tr 5:1 for all groups of strains. The FC-A strains, unlike the R strains, gave low values of *r* between Su and Tr and between Tr and Su/Tr 5:1. A possible explanation for this is given.

Finally, a hypothesis is put forward to explain the fact that no significant changes were found in the sensitivity of Dutch gonococcal strains to Am, P, T, Ri, Sp, Su, and Tr in the period from 1967 to 1972, while the S strains (which may be regarded as imported) showed a significantly higher percentage of strains relatively resistant to Am, P, T, Ri, and Tr.

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